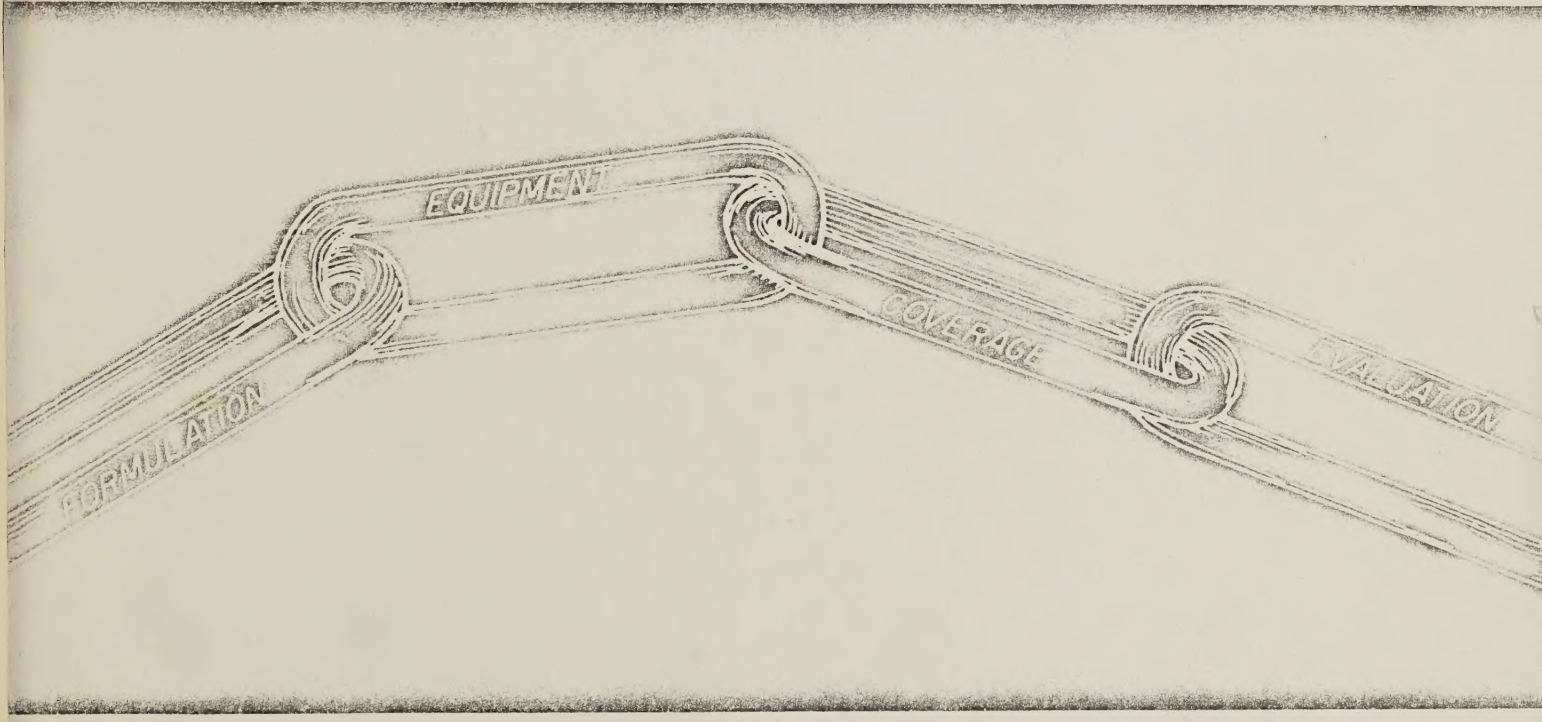
UNITED STATES DEPARTMENT OF AGRICULTURE, FOREST
SERVICE, AND AGRICULTURAL RESEARCH SERVICE

PROGRESS REPORT

BIOLOGICAL EVALUATION OF HELICOPTER SPRAY EQUIPMENT FOR APPLYING POLYHEDROSIS VIRUS TO CONTROL THE DOUGLAS-FIR TUSSOCK MOTH

by

Bohdan Maksymiuk, P. A. Boving, R. D. Orchard, and R. G. Winterfeld



Pacific Northwest Forest and Range Experiment Station
Forestry Sciences Laboratory, Corvallis, Oregon
and
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

SUMMARY

A series of helicopter spray tests were conducted on an open-ground spray test site near Corvallis, Oregon. These tests were a part of an overall effort to develop an operational method for control of the Douglas-fir tussock moth, Hemerocampa pseudotsugata McD., with a polyhedrosis virus. The main purpose of the test was to determine the effect of various spray atomizations and application rates on larval mortality.

Two spray systems were tested. The ultra low volume (ULV) spray system was calibrated to produce a spray atomization of 106 microns mass median diameter (mmd) and an application rate of 0.2 gallons per acre (gpa). The low volume (LV) spray system was calibrated to produce spray atomization of 355 microns mmd and application rates of 1.0 and 2.0 gpa. Mortalities were recorded until pupation. Distribution of spray deposit across the spray swath was determined.

Highlights of results are as follows:

1. It was discovered that application rate can be reduced tenfold by using ULV spray system without decreasing insect mortality. Furthermore, only one-half the amount of virus was used for the application rate of 0.2 gpa as compared to either 2.0 and 1.0 gpa. This provided an important lead for the design of an elaborate field test in the forest near Mt. Hebo, Oregon, that is reported elsewhere.

2. All treatments were satisfactory for aerial application of virus against the Douglas-fir tussock moth. However, ULV application showed the most potential for field use considering biological

effectiveness, spray distribution, plant coverage, simplicity of equipment, low weight of spray load for the helicopter, and reduction in cost for spray formulation and field application. The end larval mortalities (until pupation) for ULV and LV treatments using both 100- and 200-foot swaths ranged from 95% to 100%.

3. Biological and physical swath widths were determined in terms of larval mortality and deposit distribution. Data show that spray swath can be increased from 100 to at least 200 feet for both ULV and LV spray equipment without significantly reducing larval mortalities. This will reduce the cost of application.

4. New plastic cards proved highly effective for qualitative and quantitative assessment of spray deposit. In the past, paper cards were used to assess spray drops and metal plates to wash out deposit for colorometric analysis. It was found that the same plastic cards can be used satisfactorily for both purposes, thus eliminating variation between sampling surfaces and reducing cost.

5. Distribution of number of polyhedra across the spray swath was determined indirectly by sensitive fluorometric analysis using fluorescent tracer in the spray formulation. Distribution of number of spray drops across the spray swath was also determined. Both of these spray deposit analyses show that ULV and LV spray equipment produced wide swaths, which is in agreement with the biological swath widths as determined by bioassay.

6. Biological and physiochemical properties of developed spray formulation were found to be highly satisfactory for aerial application.

II. OBJECTIVES

The main purpose of the open-ground spray test was to evaluate spray equipment, develop laboratory and field techniques for aerial application, and to assess water-base sprays. Specific objectives were as follows:

1. Evaluate biological effectiveness of the ultra low and the low volume spray systems based on larval mortalities.

2. Develop and test a method for qualitative spray deposit assessment.

3. Confirm the performance of a new water-base virus spray formulation.

4. Evaluate new plastic cards for quantitative and qualitative spray deposit assessment.

III. MATERIALS AND METHODS

A. Area.--A spray site for aerial application of microbial insecticides is located about 8 miles north of Corvallis. It is a part of a 96-acre tract recently acquired by the Pacific Northwest Forest and Range Experiment Station.

B. Equipment.--A Bell 47D-1 helicopter, equipped with the ultra low and low volume spray systems, was used to conduct tests. The ultra low volume spray system was calibrated to deliver 0.2 gpa and spray atomization of 106 microns mmd.[2/] Bals turbair spinning nozzles

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[2/] Mass median diameter (mmd.) is the drop diameter satisfying the requirement that half the volume is in drops smaller and half is in drops larger than it.

were used as atomizing devices. The low volume spray system was calibrated to deliver 1.0 and 2.0 gpa by varying the number of nozzles without changing spray atomization. Spray atomization was 355 microns mmd. Flat spray nozzles 8003 were used to atomize spray. Detailed description of spray equipment and related information is reported by Boving et al. (1968).

C. Formulation.--A recently developed water-base spray formulation for aerial application of microbial insecticides was used to apply the virus (Maksymiuk, 1968). This formulation contained a fluorescent tracer for spray deposit assessment. Final pH was 7.0 which is optimal for virus stability. Ingredients of the spray formulation and their sources are given in Table 1.[3/]

Table 1.--Ingredients of spray formulation

Ingredients	Amount to make 100 gallons	Percent	Source
Cellosize, HEC QP-100M	757 g	0.2	Union Carbide Corp.
Antifoam, H-10 emulsion	379 ml	0.1	Dow Corning Corp.
Monawet, Mo-70	1893 ml	0.5	Mona Industries
Hycar, 1872 x 6	3785 ml	1.0	B. F. Goodrich Co.
Brilliant sulpho flavine FFA	379 g	0.1	General Aniline and Film Corp.

[3/] Mention of products and their sources does not imply endorsement by the USDA.

D. Virus.--A supply of virus was produced commercially on contract by Nutrilite Corporation. This product consisted of ground diseased larvae containing nucleopolyhedrosis virus of the Douglas-fir tussock moth. Purity, concentration of polyhedra, and toxicity-pathogenicity for mice were determined (Martignoni, 1967).

E. Insects.--About 4,000 larvae of the Douglas-fir tussock moth were mass-reared from laboratory-produced eggs without noticeable incidence of disease. The insect culture originated from eggs collected near Goose Lake, California, on November 1965. The larvae were reared on an artificial diet. Ingredients of the diet and the method of preparation is given in Appendix A. Procedure for egg sterilization is given in an Appendix B. Late second and early third instar larvae (15-19 days old) were used for virus bioassay and for the control in all tests.

F. Trees.--Potted and greenhouse-grown Douglas-fir trees, Pseudotsuga menziesii, were used for bioassay. By certain greenhouse techniques, it was possible to obtain a flush of new growth and to time it with the schedule of spray tests.

G. Treatments.--The following variables were tested:

a) Ultra low volume spray equipment--application rate of 0.2 gpa, spray atomization 106 microns mmd and virus concentration of 25×10^9 polyhedra per acre, assuming 100-foot swath.

b) Low volume spray equipment--with application rates of 1.0 and 2.0 gpa using the same spray atomization of 355 microns mmd and virus concentration of 50×10^9 polyhedra per acre, assuming 100-foot swath.

H. Tests.--Spray tests were conducted on June 6, 1967. For all spray tests a similar procedure was used as follows:

a) Forty-one potted plants were placed in line at 10-foot intervals.

b) Two plastic spray deposit assessment cards were also placed in line on opposite sides of each potted plant.

c) Additional cards (two per sampling station) were placed at 10-foot intervals to extend spray deposit sampling line (Fig. 1).

d) The virus spray formulation was applied by a helicopter flying about 50 feet above and across the sampling line at a speed of 45 miles per hour (mph).

e) After about 15 minutes of waiting for the spray to settle down, perforated plastic bags were slipped over all potted plants and they were transported to the laboratory for insect bioassay.

f) The spray deposit cards were removed from the sampling stations and placed in slotted boxes and transported to the laboratory for spray deposit assessment.

I. Bioassay.--In the laboratory, incisions were made in the plastic bags, 10 larvae were placed on the foliage of each of the potted plants, and the incisions were sealed with masking tape (Maksymiuk and Orchard, 1968). In a similar manner, controls were set up before treatments to avoid possible contamination of foliage with virus. After 10 days, larval mortality was recorded and all live larvae were transferred to artificial medium (Appendix). Five or less larvae were placed in each Petri dish. Mortality was recorded daily until pupation. Smears of all dead larvae were examined under a phase microscope for presence

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[illegible] similar [illegible]

[illegible] forty-one [illegible]

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a possible contamination of [illegible] with [illegible]

[illegible]

[illegible] five or less larvae [illegible] in each

[illegible]

[illegible]

and relative amount of polyhedra and other possible pathogens in order to diagnose the cause of mortality. Larval mortalities were determined for each sampling station. Biological swath widths were determined based on larval mortality at these stations. This way, it was possible to establish a mean mortality for a given swath width (100, 200, and 300 feet) for various treatments at given time intervals.

J. Deposit.--Two plastic cards (4 x 5 inches) attached individually to aluminum supporting plates were placed at 10-foot intervals to sample spray deposit across the swath (Maksymiuk, 1968b). Sprayed cards with aluminum supporting plates were placed in slotted boxes and transported to the laboratory. The spray formulation contained a fluorescent tracer (0.1% of brilliant sulfo flavine FFA) for deposit analysis. The number of polyhedra was determined by fluorometric analysis. This involved removing the spray deposit from the plastic cards with 95% ethanol, obtaining a reading in the fluorometer and comparing it with the previously developed standard curve showing the relationship between reading and concentration of fluorescent tracer. A Turner fluorometer, Model 111, was used. It was equipped with a high sensitive door and a combination of two filters (primary filter No. 405 and secondary filter No. 2A-12).

The number of spray drops, regardless of size, was determined on two cards (4 square centimeters per card) that were placed at 10-foot intervals across the spray swath. Drop spots were counted under a dissecting microscope using ultraviolet light for illumination to see the fluorescent deposit.

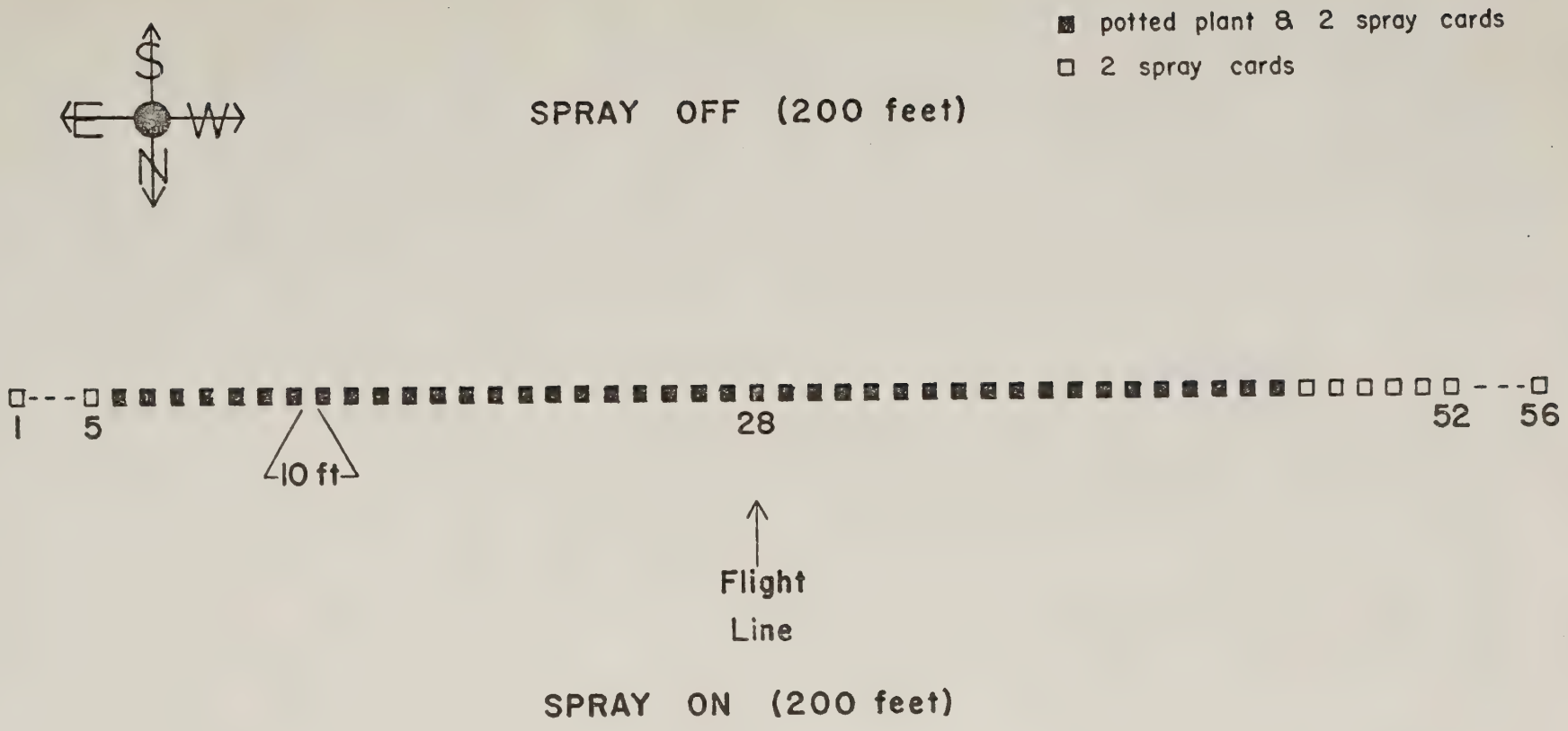


Fig. 1.--Layout for sampling of spray deposit to determine the biological swath width and spray deposit distribution.

IV. METEOROLOGICAL

Meteorological conditions for all test flights were recorded (Table 2).

Table 2.--Meteorological data

Flight no.	Time	Wind		Temp. °F	R.H., %	Remarks
		Speed	Dir.			
1	6:40 a.m.	3 mph	South	53	77	Gusty
2	11:14 a.m.	3 mph	South	62	58	Overcast
3	12:00 noon	3 mph	South	65	55	Overcast
4	12:45 p.m.	1 mph	SE-E	70	50	Overcast

V. RESULTS AND DISCUSSION

A. Biological Evaluation of Ultra Low and Low Volume Spray Equipment

Corrected larval mortality for all treatments, for various swath widths and time intervals, is summarized in Tables 3 and 4. According to these data, the feasibility of ultra low volume application of microbial insecticides using fine spray atomization (106 microns mmd) was demonstrated for the first time. It was found that the application rate can be reduced tenfold (from 2.0 to 0.2 gpa) without reducing insect mortality even with a 50% reduction of virus dose (Tables 3 and 4).

Uncorrected cumulative daily larval mortalities (all treatments and control) for 100- and 200-foot swath widths are presented in Figs. 3 and 4, respectively. In general, the larval mortality trend was similar

for all treatments when either 100- or 200-foot swath widths were used. However, there was less difference in larval mortalities among different treatments when a 100-foot swath width was used as compared to the mortalities with a 200-foot swath width.

Biologically effective swath widths and variations of corrected larval mortalities (in 14 days) across the spray swaths are graphed for all test flights (Figs. 4-7). All treatments show biologically effective swath widths up to and including 200 feet. It was found that the performance of ultra low volume and low volume spray equipment using a new spray formulation was superior to the previously used spray equipment for applying microbial insecticides in water-base carriers.

Distribution of uncorrected larval mortalities across the spray swath for 10, 20, 30, and 40 days for all treatments are presented in frequency distribution histograms (Figs. 8-11). It is evident that the degree and uniformity of larval mortalities were increasing with the time.

Table 3.--Summary of early larval mortalities for all treatments

Treatments	Flight no.	Swath width, feet	Polyhedra per acre	No. of trees	No. of larvae	Cor. larval mort., % - Days 10	14	21
0.2 gpa 106 microns	1	100	25.00×10^9	11	119	30 ± 11[a]	79 ± 13	97 ± 5
		200	12.50×10^9	21	217	28 ± 8	78 ± 8	95 ± 4
		300	8.33×10^9	31	317	23 ± 7	66 ± 9	84 ± 8
0.2 gpa 106 microns	2	100	25.00×10^9	11	111	22 ± 8	56 ± 12	87 ± 8
		200	12.50×10^9	21	208	20 ± 4	50 ± 8	78 ± 8
		300	8.33×10^9	31	308	17 ± 4	44 ± 7	72 ± 8
1.0 gpa 355 microns	4	100	50.00×10^9	11	109	31 ± 13	71 ± 10	95 ± 6
		200	25.00×10^9	21	203	31 ± 9	60 ± 12	84 ± 13
2.0 gpa 355 microns	3	100	50.00×10^9	11	112	21 ± 10	66 ± 13	94 ± 6
		200	25.00×10^9	21	209	31 ± 10	71 ± 9	93 ± 4
		300	16.67×10^9	31	304	28 ± 8	65 ± 9	88 ± 6
		CONTROL		25	249	1 ± 1	3 ± 2	13 ± 4

[a]/Standard error of the mean estimate at the 95% probability level (confidence limits) is given throughout this report.

Table 4.--Summary of end larval mortalities for all treatments

Treatments	Flight no.	Swath width, ft.	No. days after treatment	Corrected mortality, %
0.2 gpa	1	100	23	98
106 microns		200	31	99
0.2 gpa	2	100	34	100
106 microns		200	61	99
1.0 gpa	4	100	28	100
355 microns		200	64	95
2.0 gpa	3	100	28	100
355 microns		200	28	100

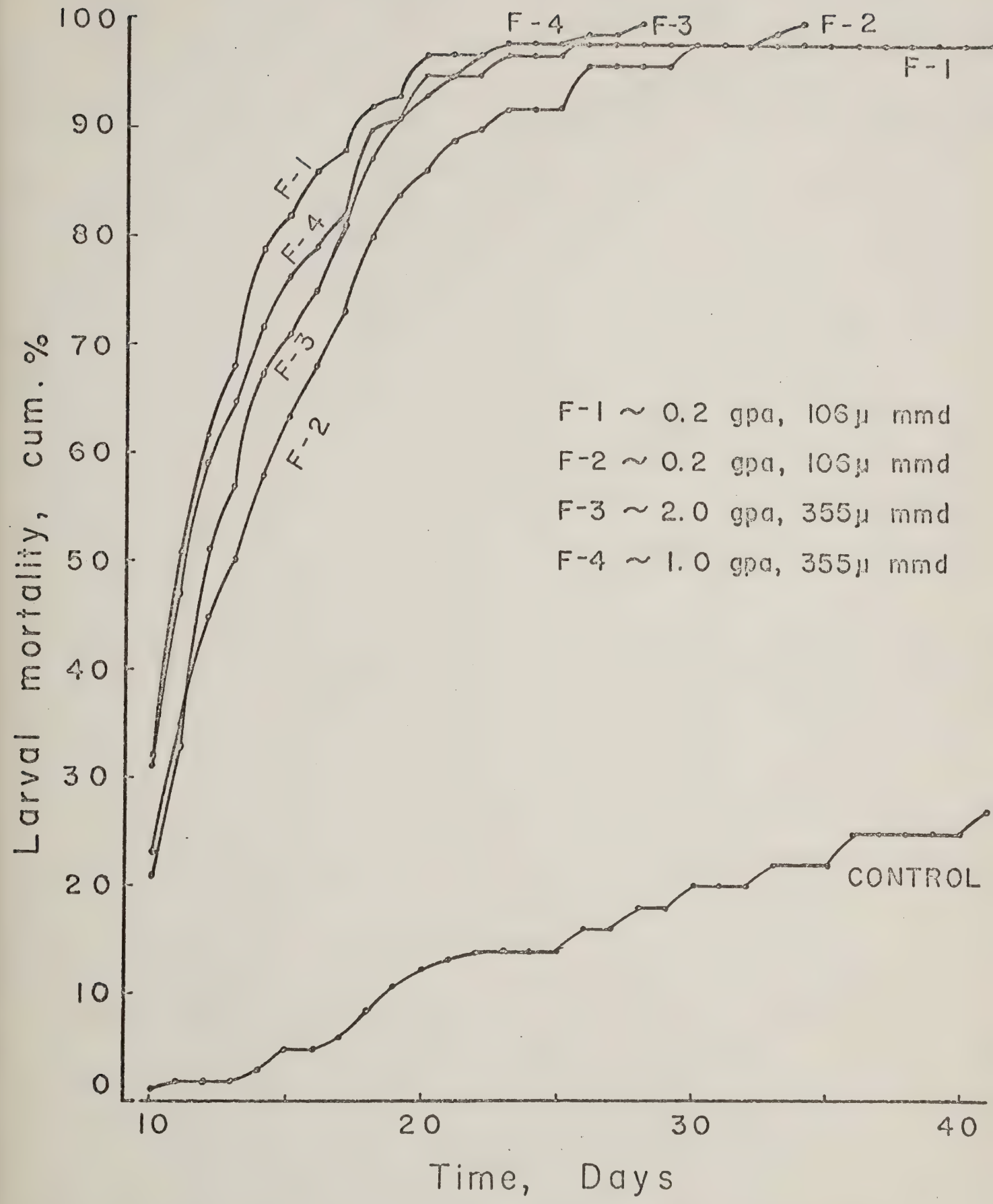


Fig. 2.--Relationships between larval mortality and time for 100-foot swath width using various application rates and spray atomizations (flights 1-4).

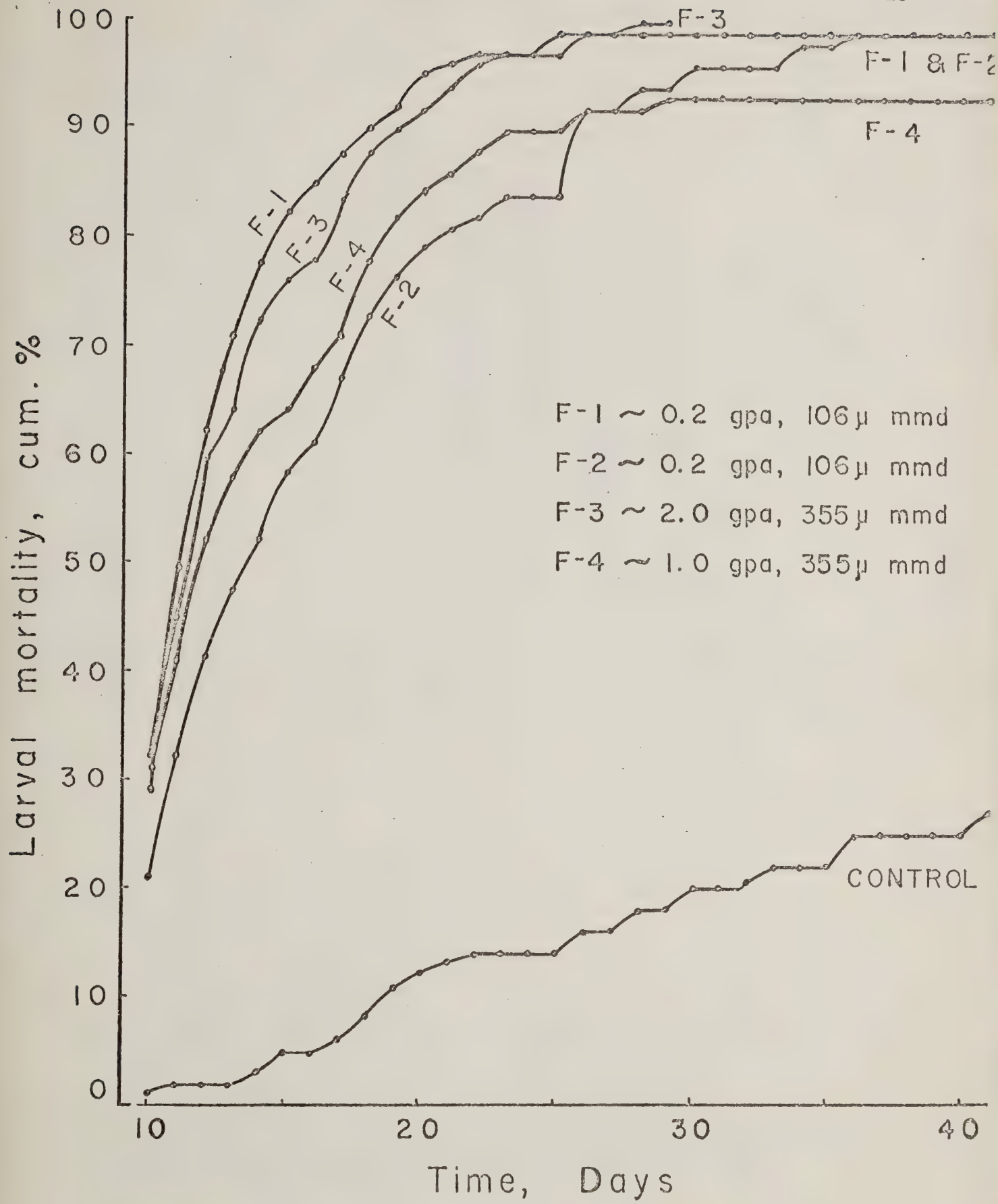


Fig. 3.--Relationships between larval mortality and time for 200-foot swath width using various application rates and spray atomizations (flights 1-4)

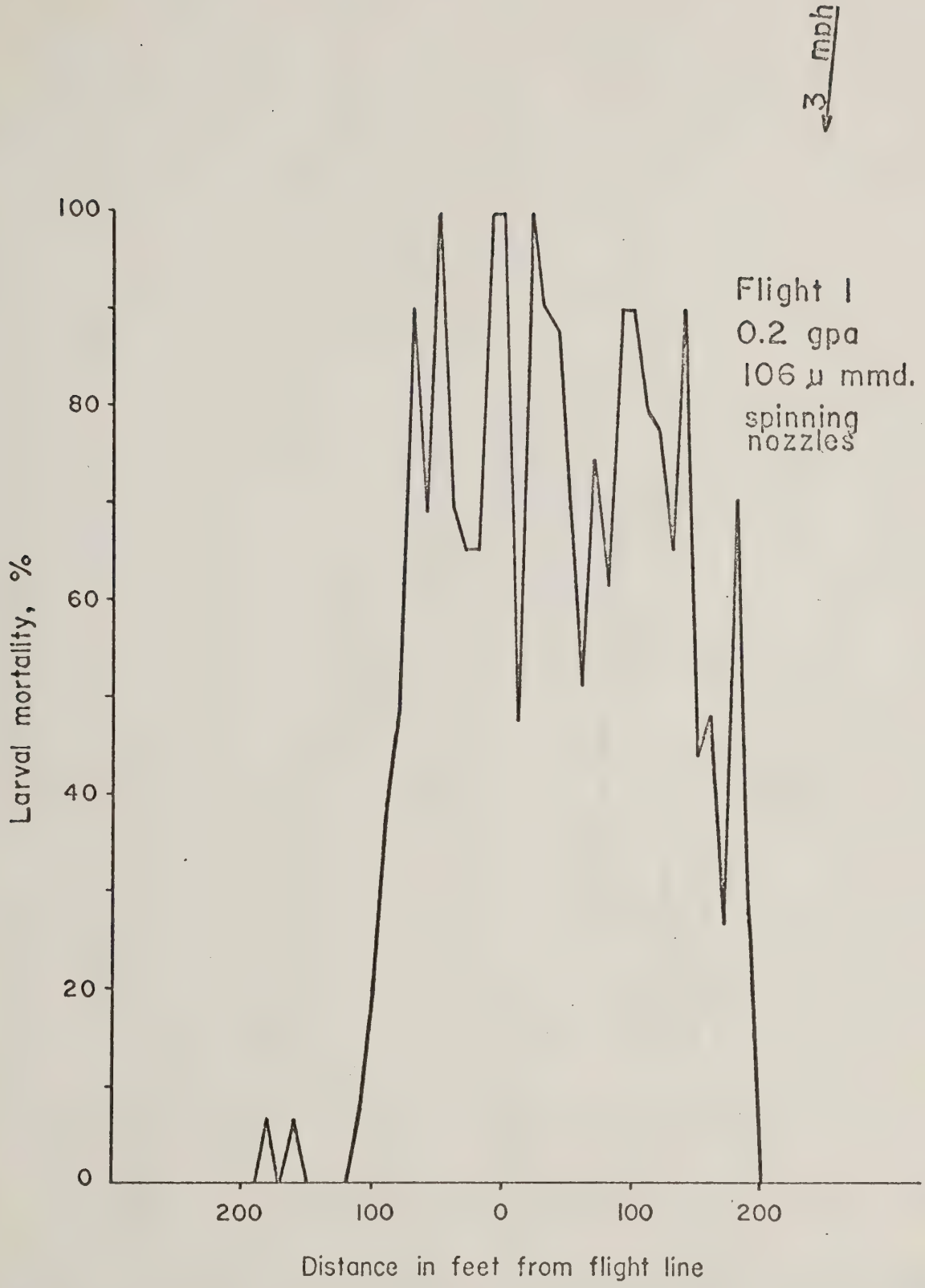


Fig. 4.--Distribution of corrected larval mortality in 14 days across the spray swath for application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 1).

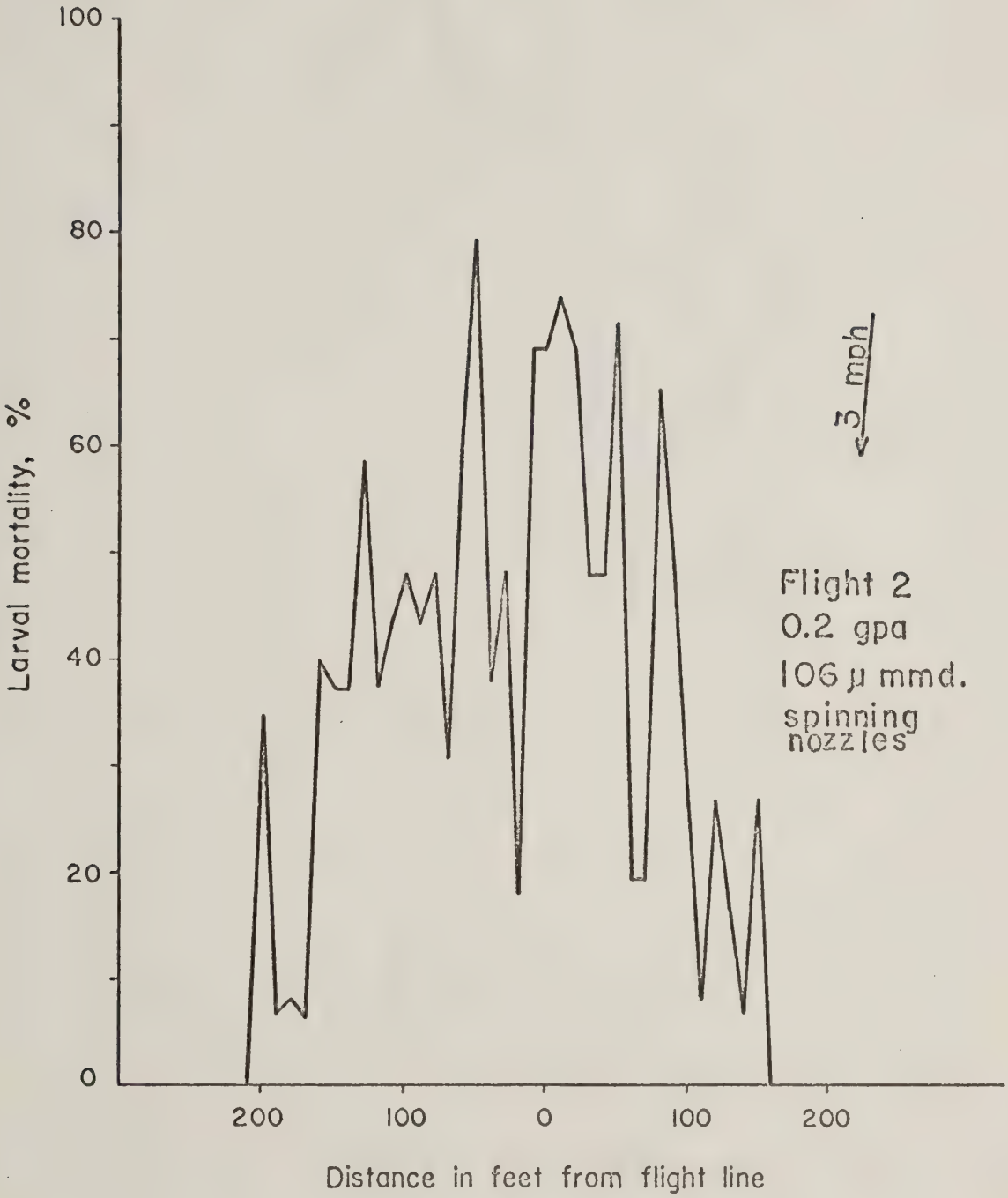


Fig. 5.--Distribution of corrected larval mortality in 14 days across the spray swath for application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 2).

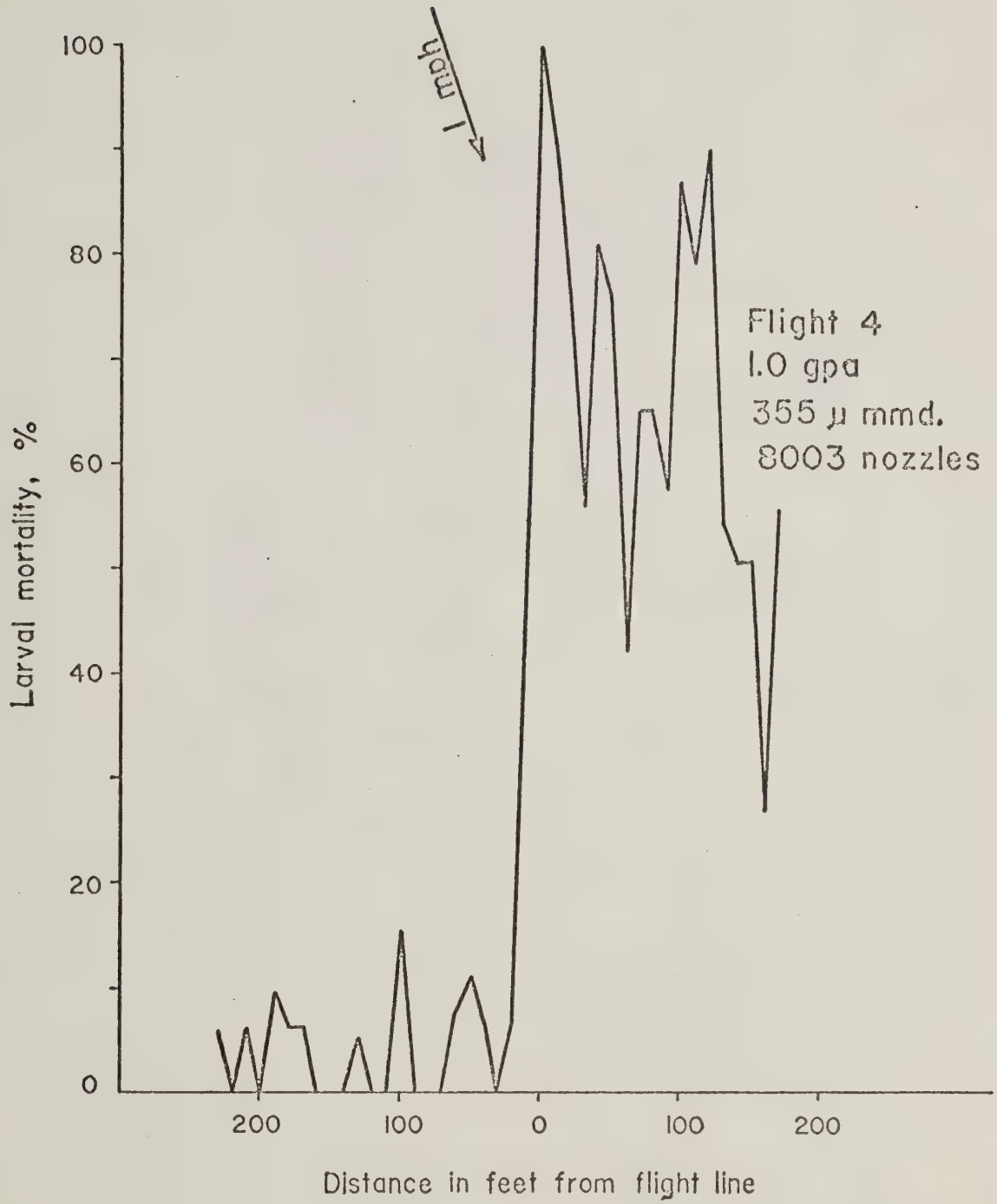
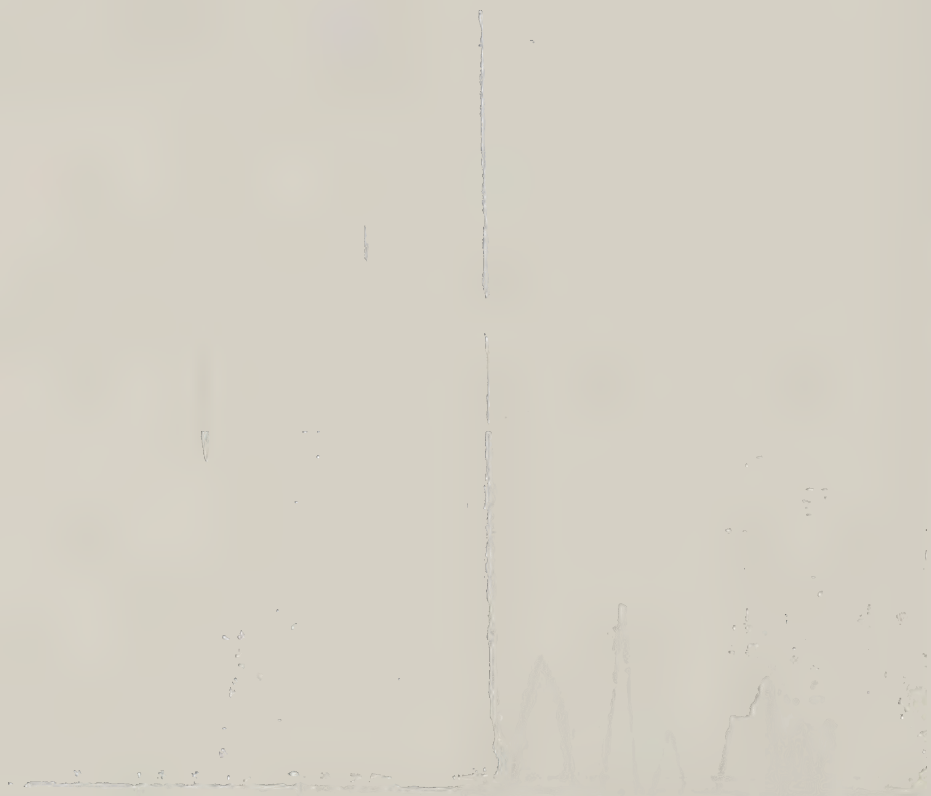


Fig. 6.--Distribution of corrected larval mortality in 14 days across the spray swath for application rate of 1.0 gpa and spray atomization of 355 microns mmd (flight 4).



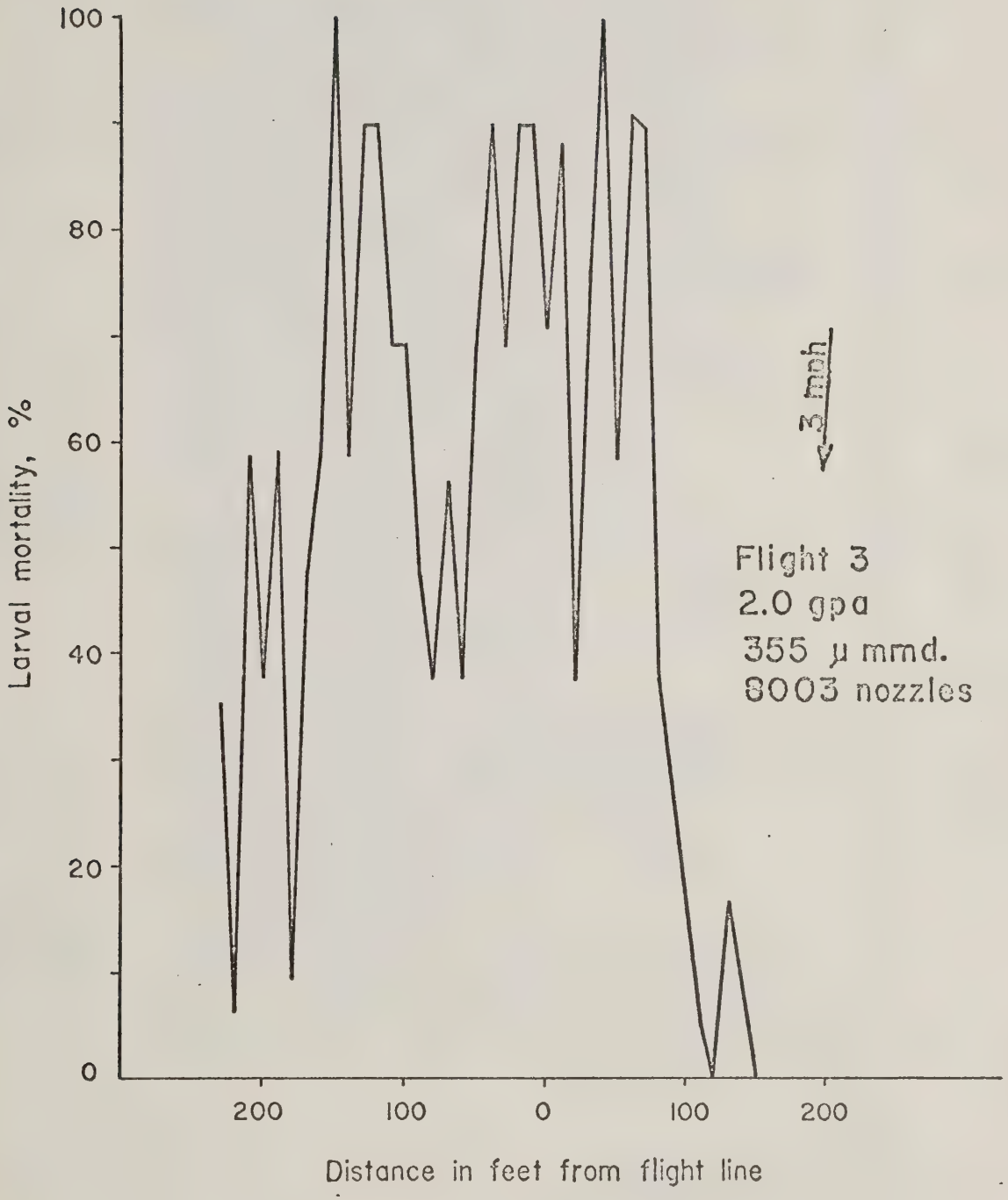


Fig. 7.--Distribution of corrected larval mortality in 14 days across the spray swath for application rate of 2.0 gpa and spray atomization of 355 microns mmd (flight 3).

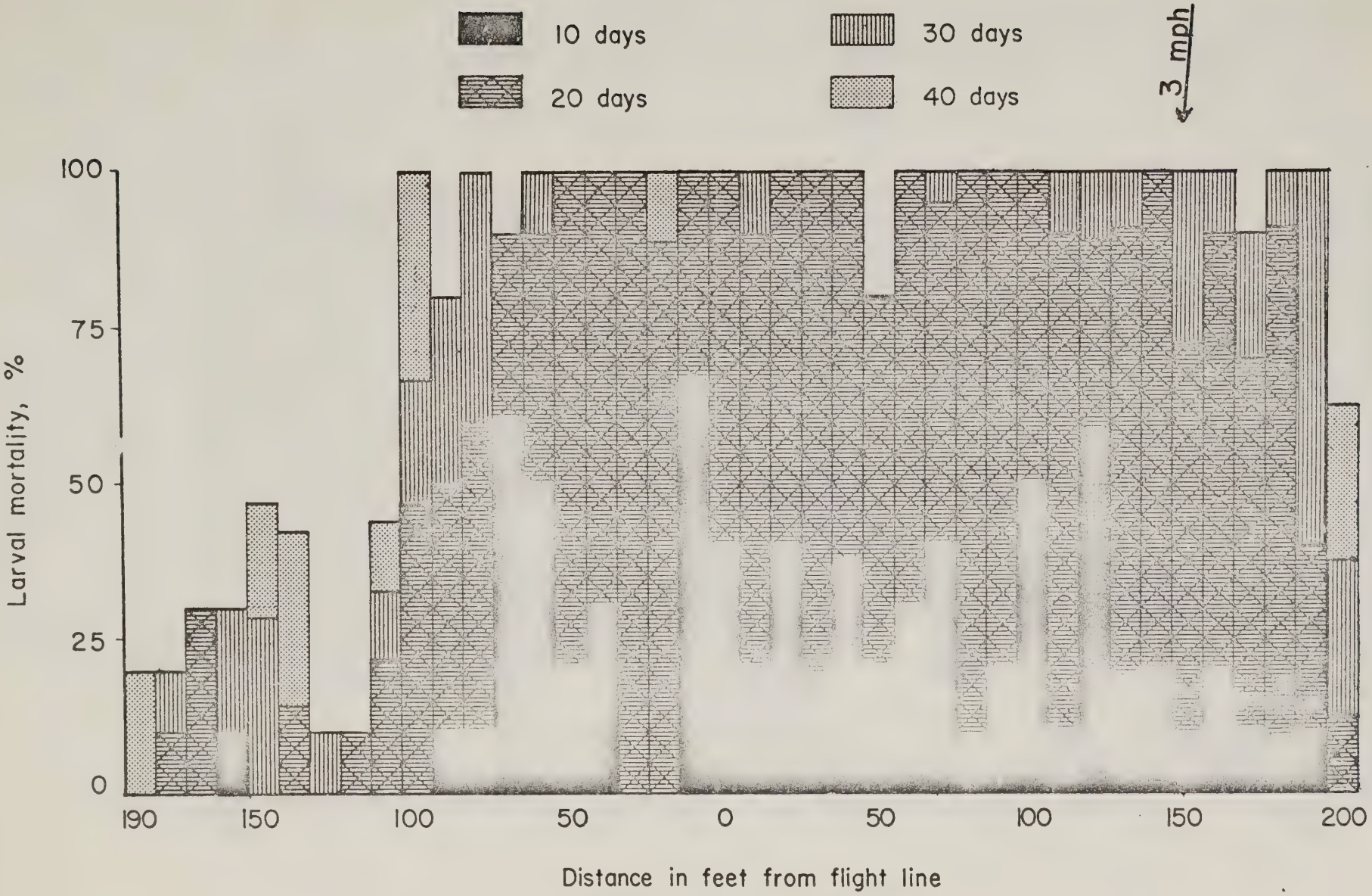


Fig. 8.--Distribution of uncorrected larval mortalities across the spray swath for different time intervals using application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 1).

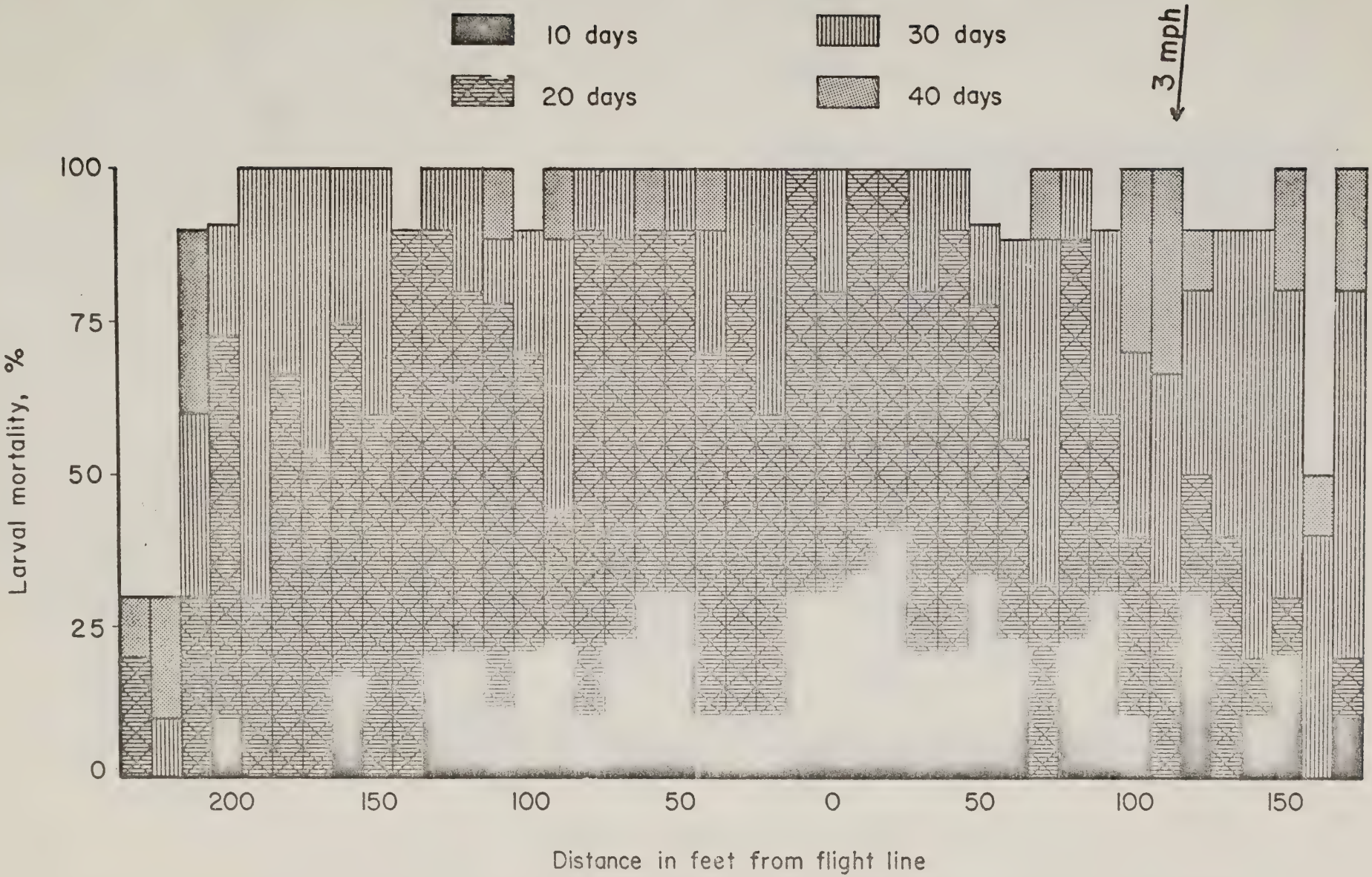


Fig. 9.--Distribution of uncorrected larval mortalities across the spray swath for different time intervals using application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 2).

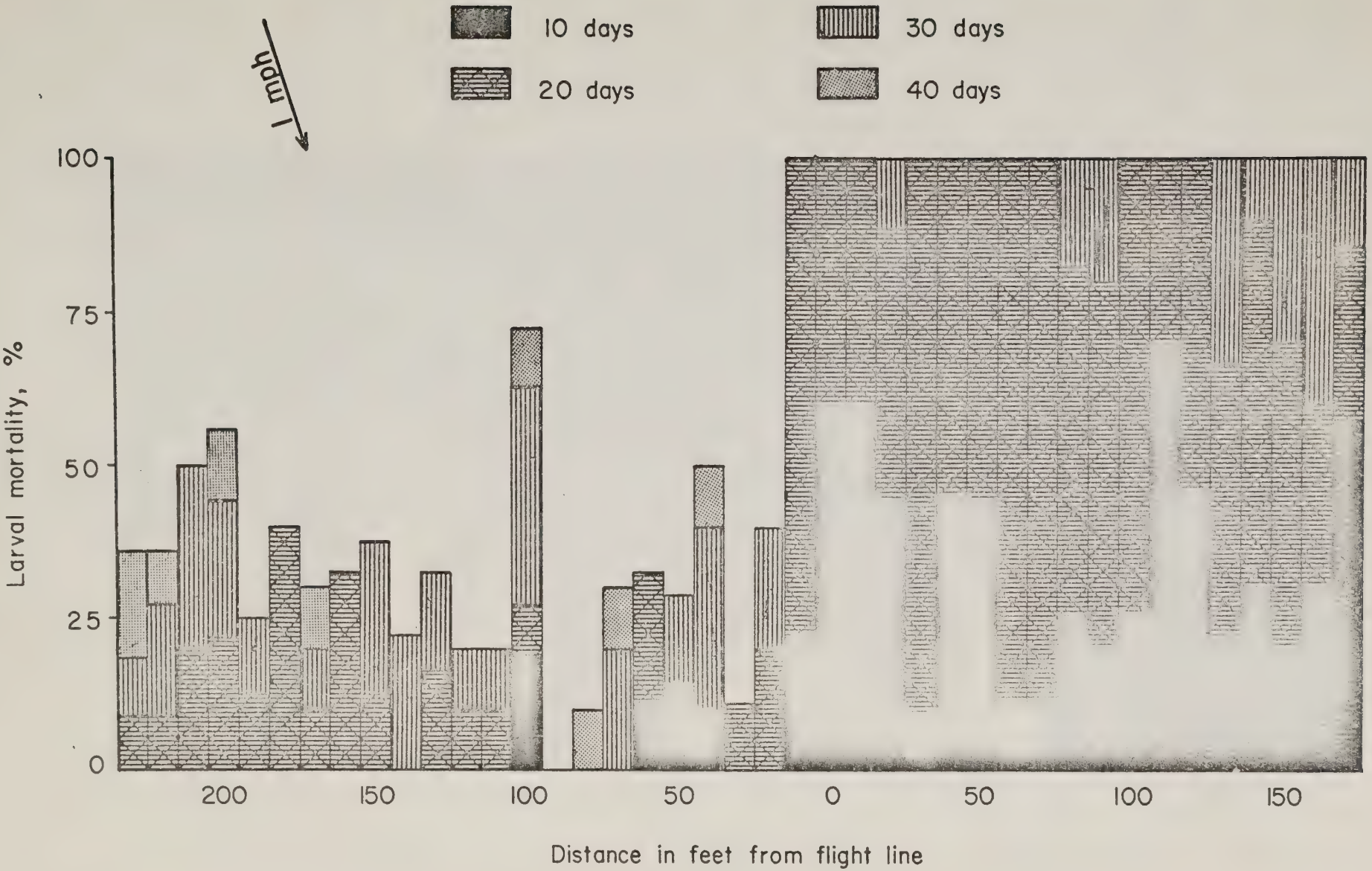


Fig. 10.--Distribution of uncorrected larval mortalities across the spray swath for different time intervals using application rate of 1.0 gpa and spray atomization of 255 microns mmd (flight 4).

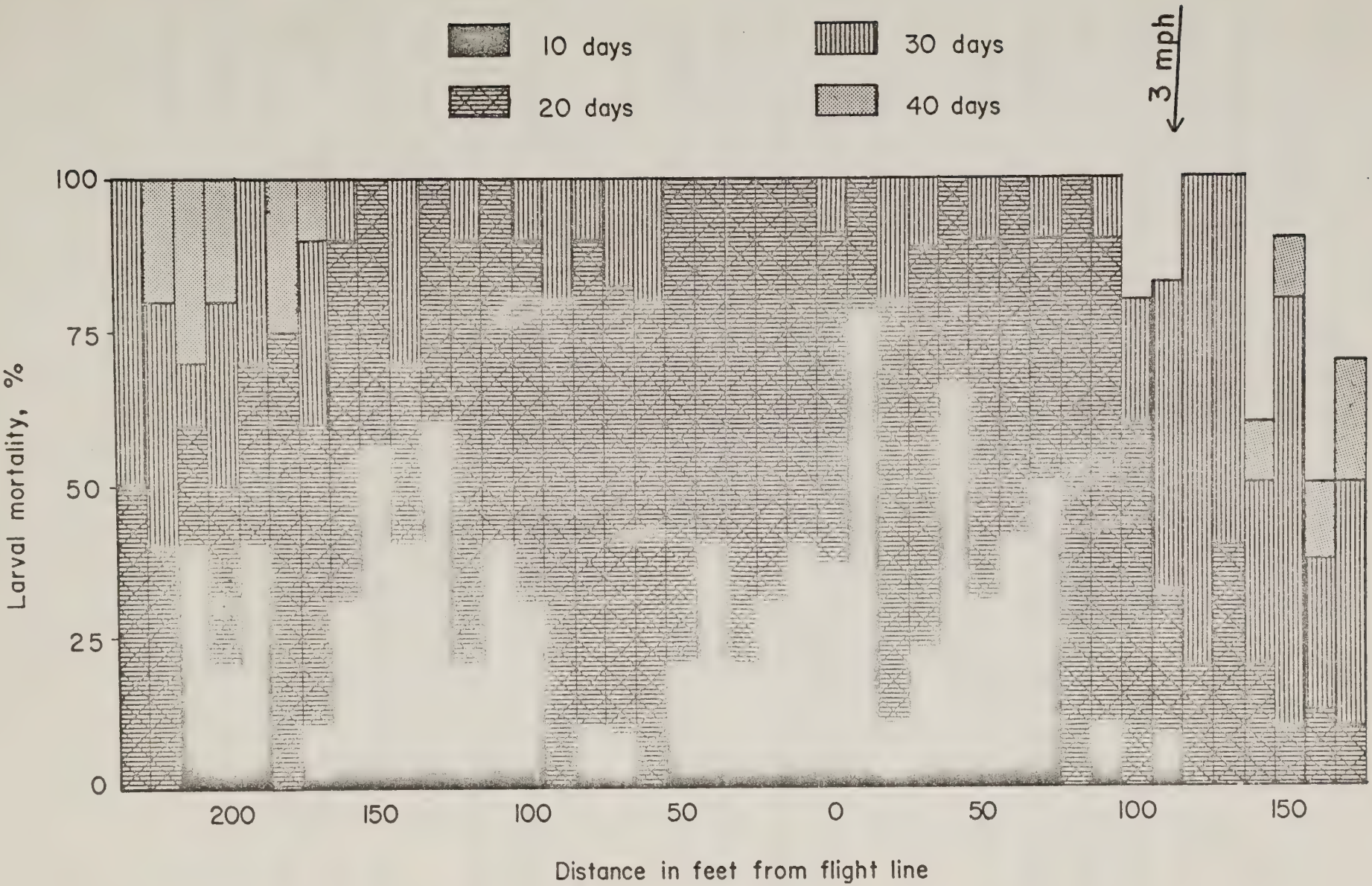


Fig. 11.--Distribution of uncorrected larval mortalities across the spray swath for different time intervals using application rate of 2.0 gpa and spray atomization of 255 microns mmd (flight 3).

B. Spray Deposit Assessment

Distribution of number of polyhedra, based on qualitative fluorometric analysis, across the spray swaths for four test flights is graphed in Figs. 12-15. These graphs show that spray deposit distribution was satisfactory for swath width up to and including 200 feet. Also, spray equipment did not produce undesirable low deposits in the middle of swaths. Fine spray atomization (106 microns mmd, 0.2 gpa) resulted in wider swath widths than coarse atomization (355 microns mmd, 1.0 or 2.0 gpa).

Distribution of spray drops based on microscopic counts across the spray swaths for four test flights is presented in Figs. 16-19. The largest number of spray drops that was recorded on plastic spray deposit cards was found in the middle of spray swaths. They were fairly evenly distributed up to 200-foot swath widths for all test flights.

The general shape of graphs (Figs. 4-7) showing larval mortalities is similar to that of graphs (Figs. 12-15) presenting the distribution of polyhedra. Also, the distribution of the number of spray drops across the swath (Figs. 16-19) is similar to the distribution of larval mortalities and number of polyhedra. This was the case for all treatments. Distribution of polyhedra across the spray swath depends on the drop size spectra produced by a given atomizing device. Detailed characteristics of drop size spectra used in this study are given elsewhere (Maksymiuk, 1968a).

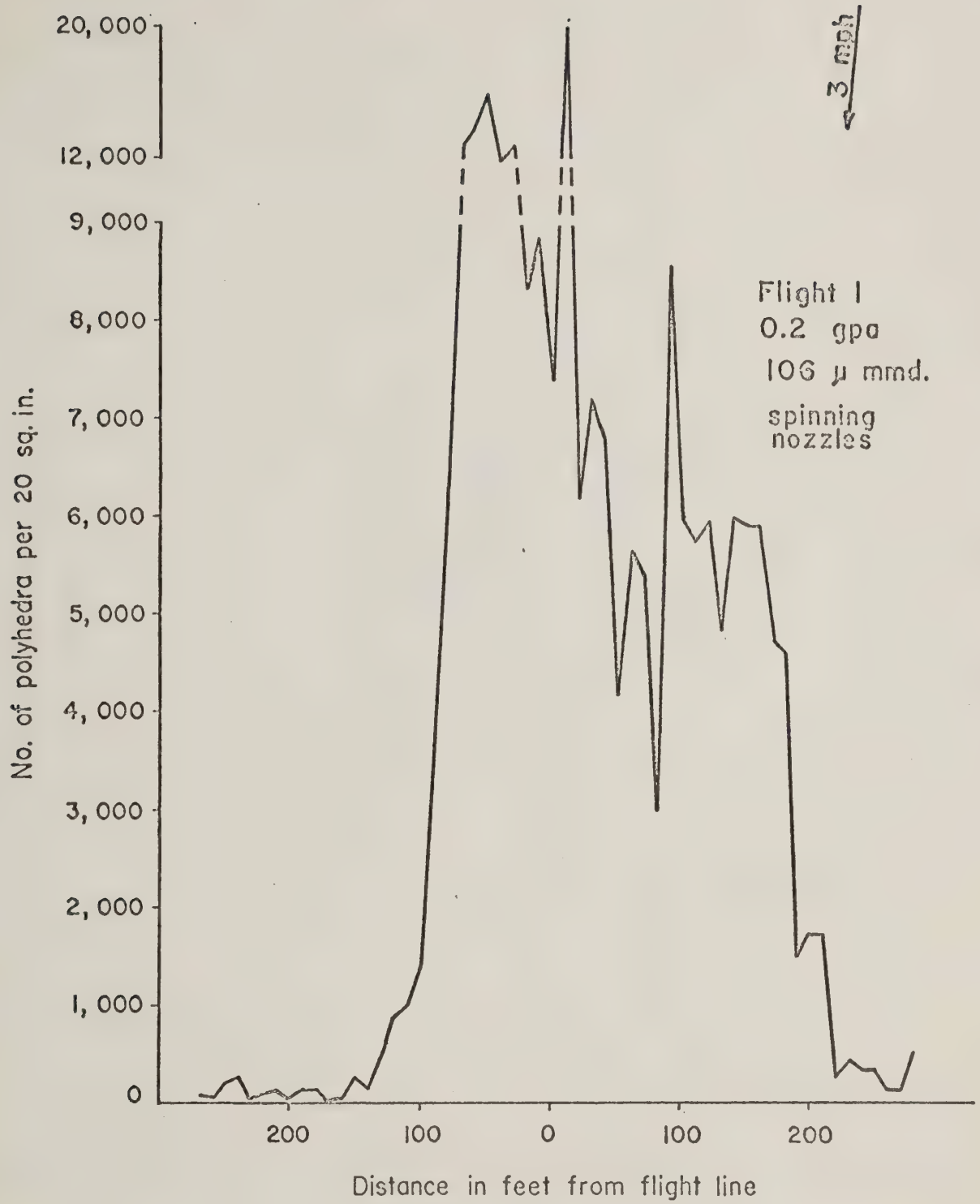


Fig. 12.--Distribution of number of polyhedra across the spray swath for application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 1).

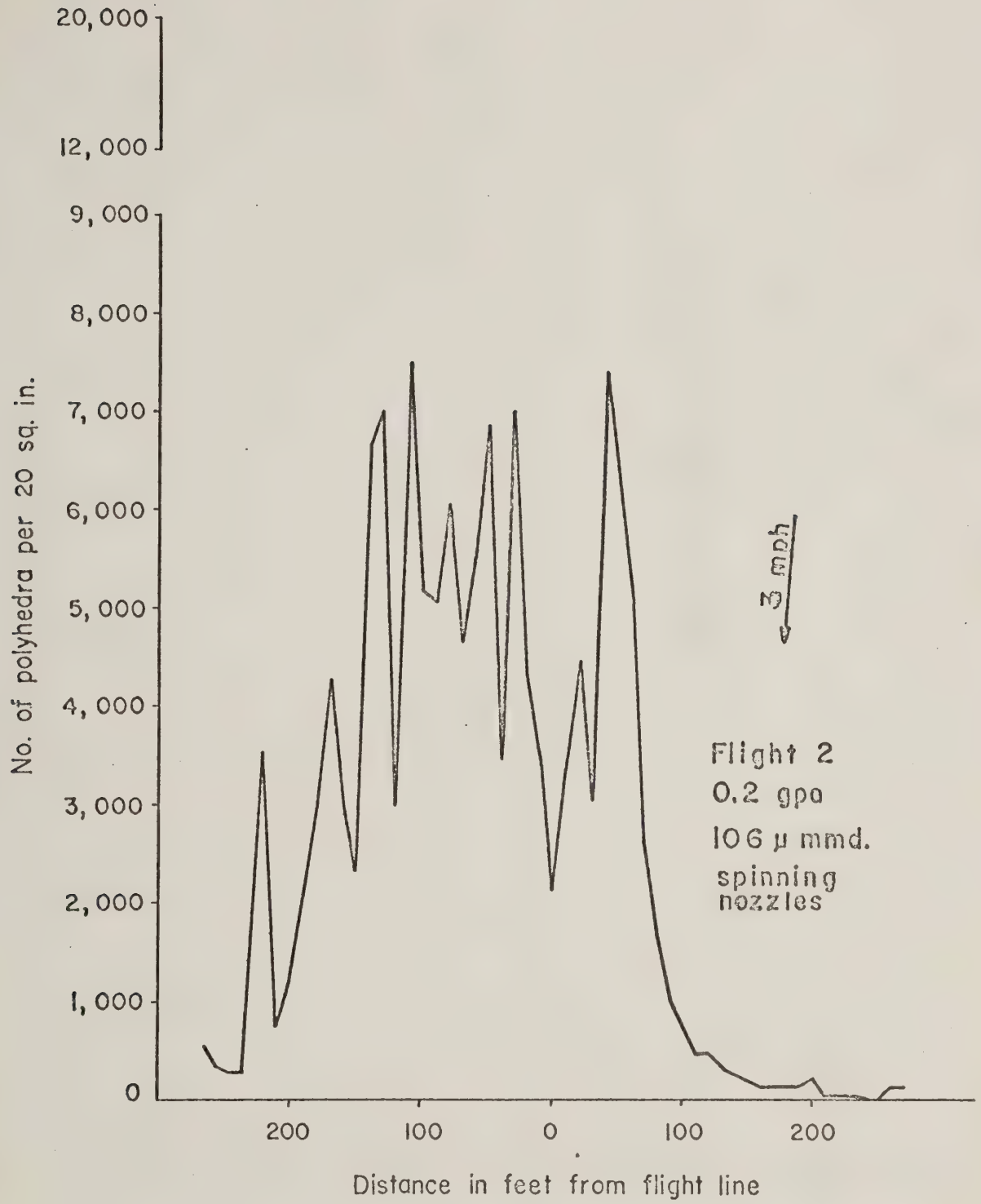


Fig. 13.--Distribution of number of polyhedra across the spray swath for application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 2).

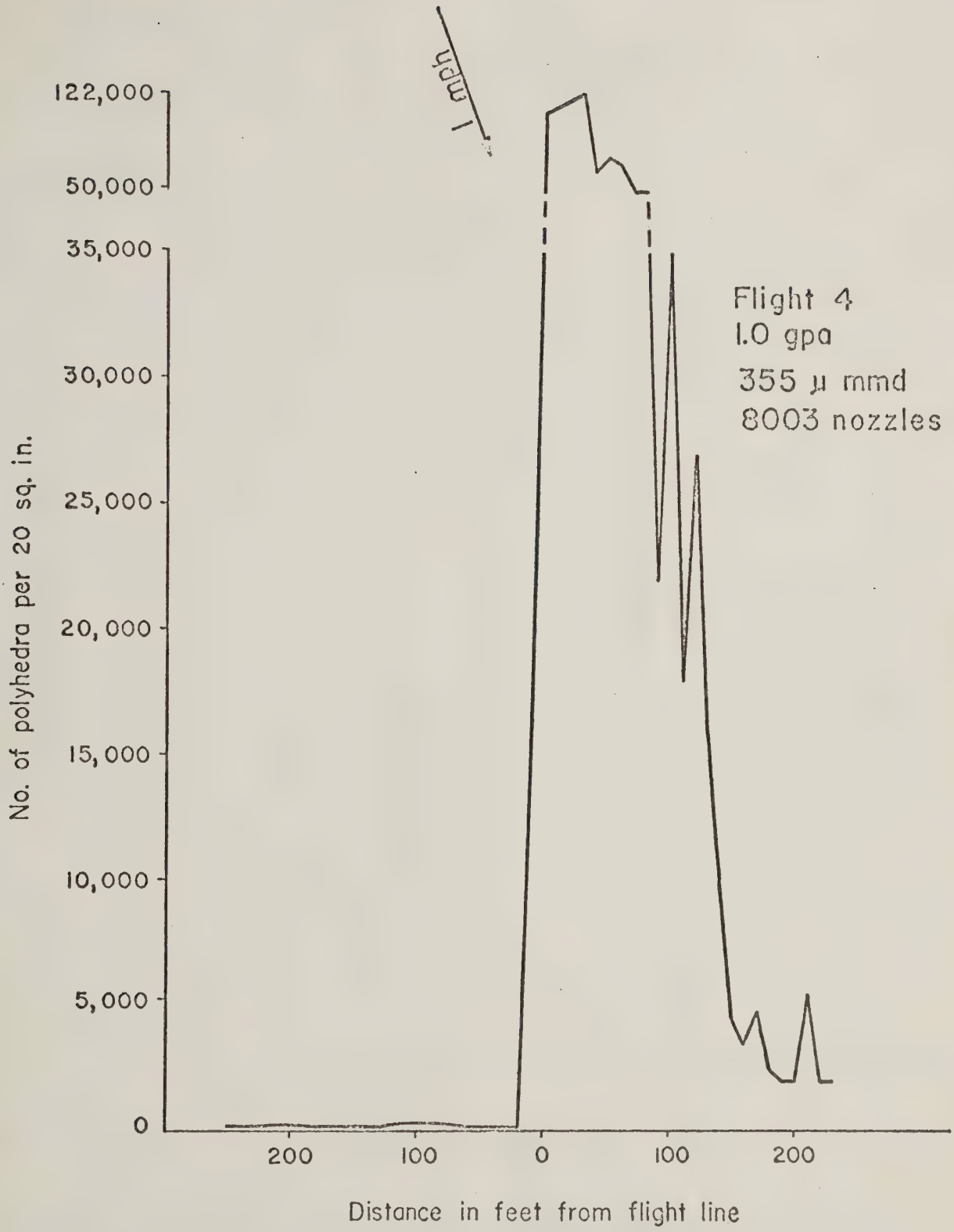


Fig. 14.--Distribution of number of polyhedra across the spray swath for application rate of 1.0 gpa and spray atomization of 355 microns mmd (flight 4).

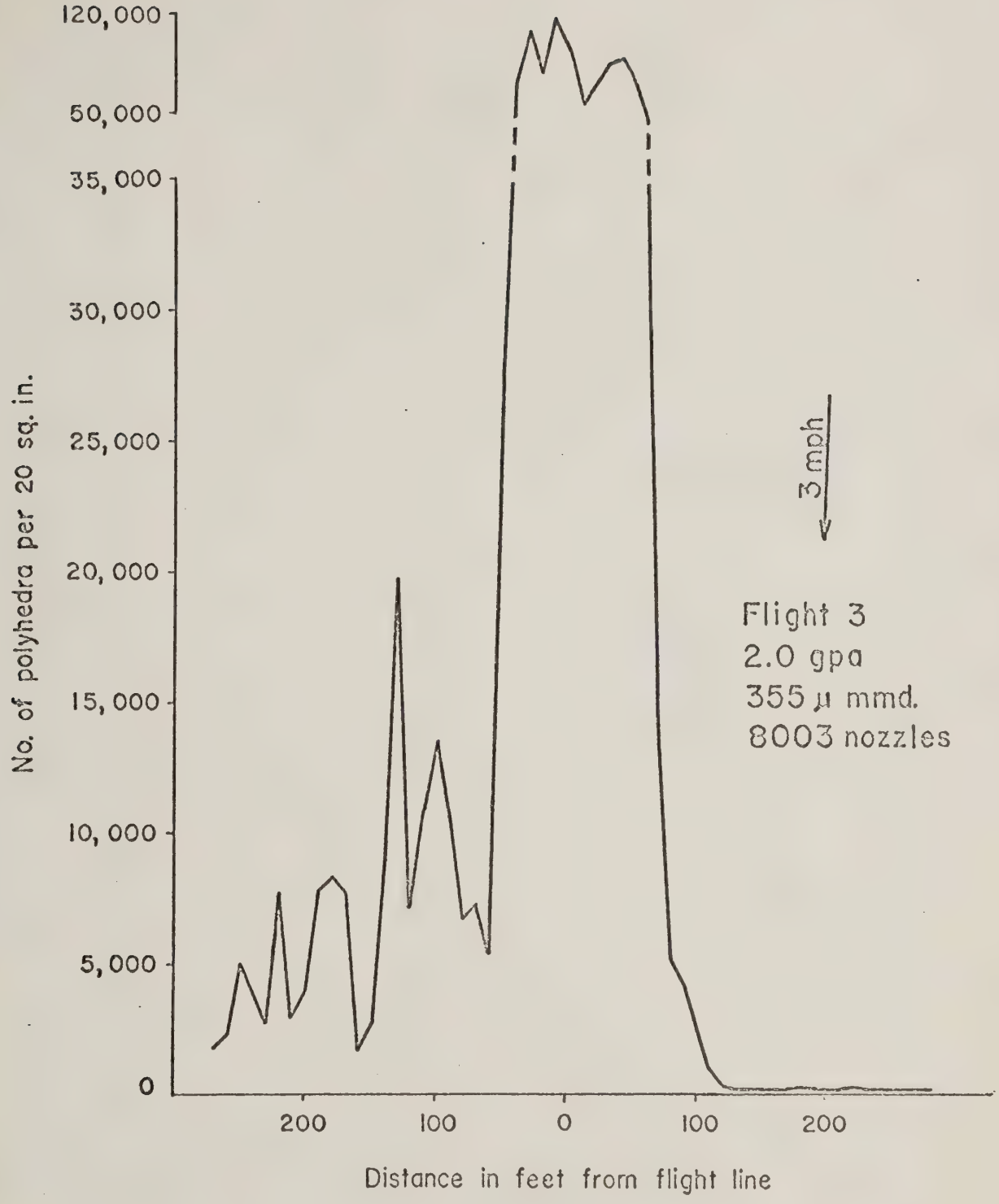


Fig. 15.--Distribution of number of polyhedra across the spray swath for application rate of 2.0 gpa and spray atomization of 355 microns mmd (flight 3).

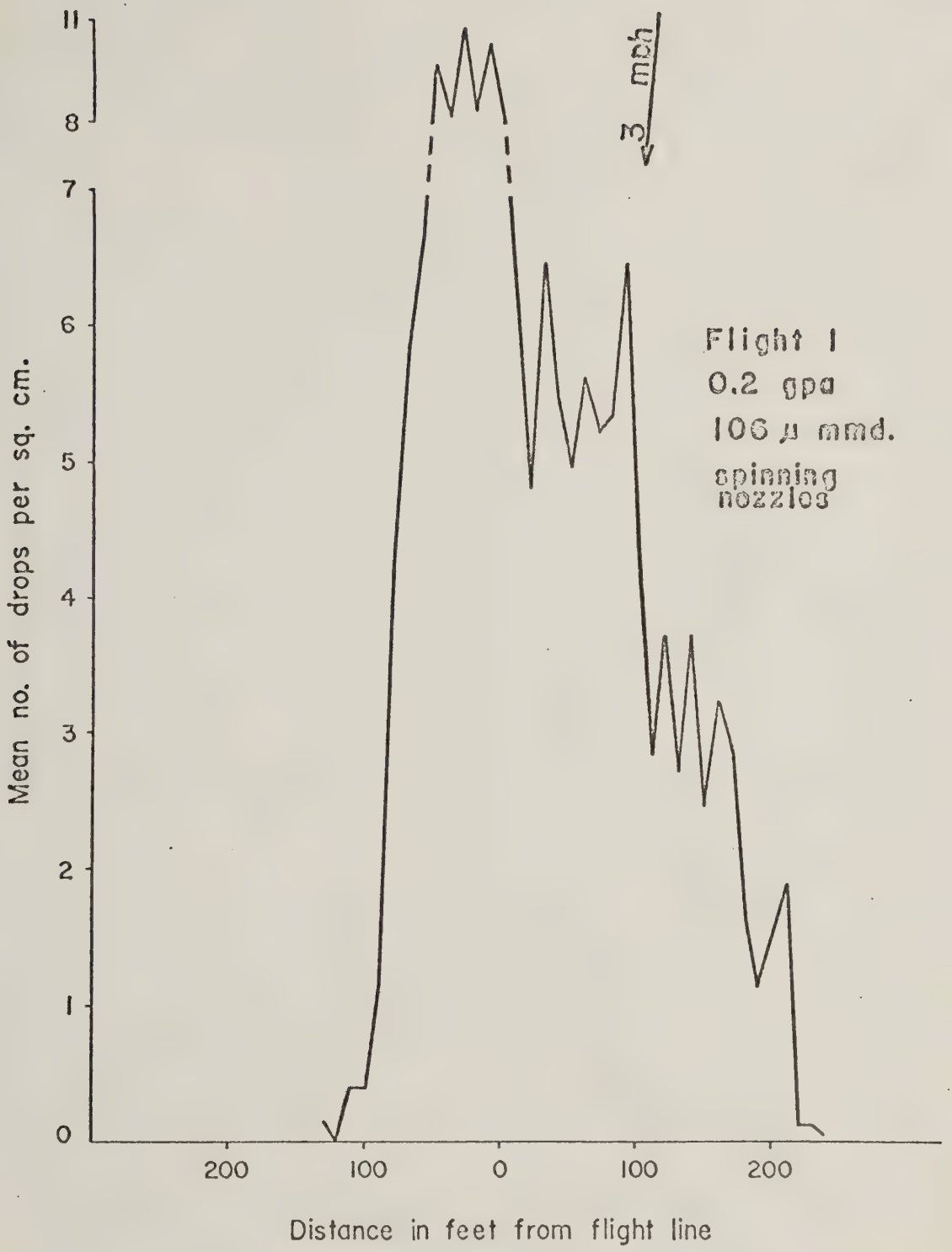


Fig. 16.--Distribution of number of spray drops across the spray swath for application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 1).

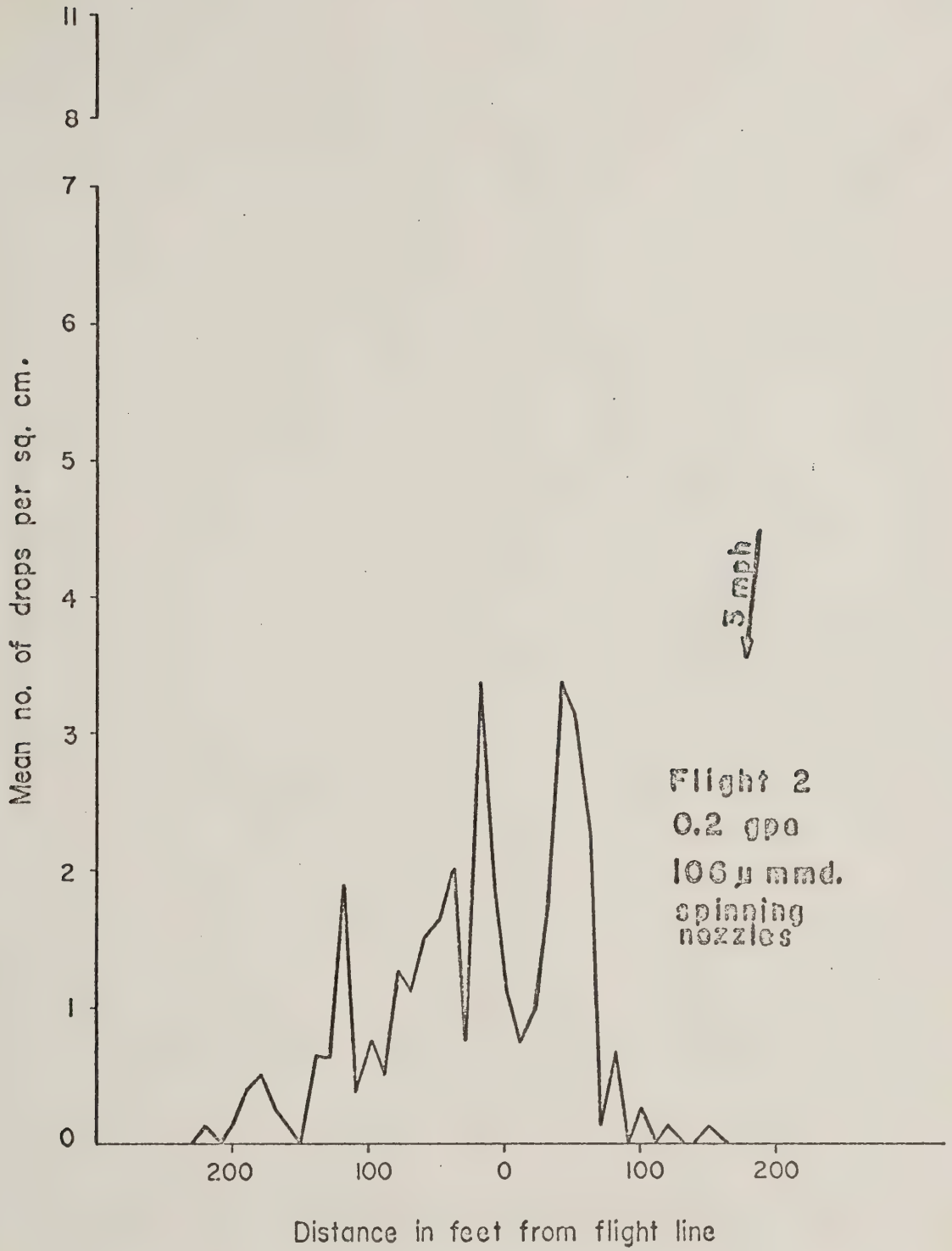


Fig. 17.--Distribution of number of spray drops across the spray swath for application rate of 0.2 gpa and spray atomization of 106 microns mmd (flight 2).

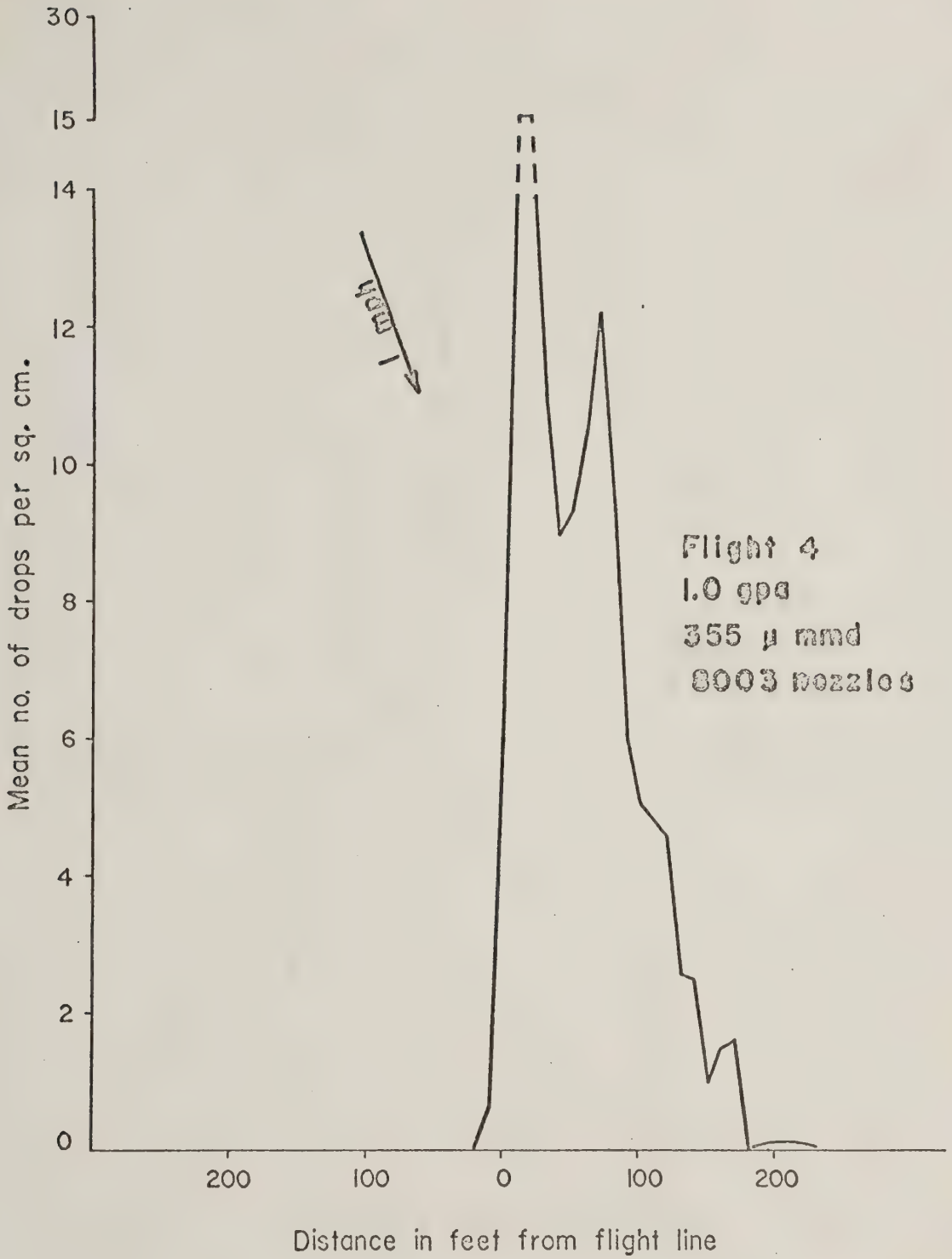


Fig. 18.--Distribution of number of spray drops across the spray swath for application rate of 1.0 gpa and spray atomization of 355 microns mmd (flight 4).

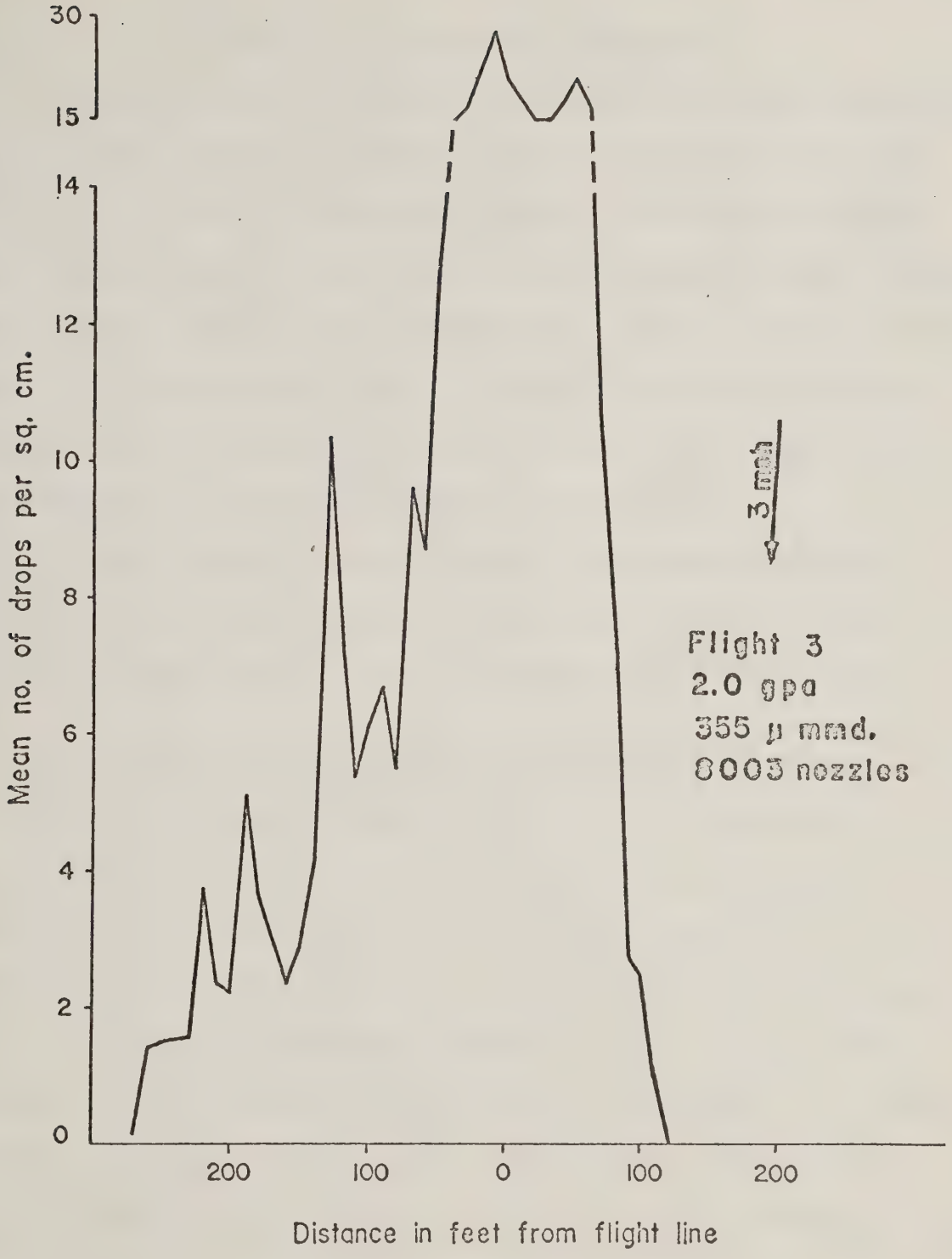


Fig. 19.--Distribution of number of spray drops across the spray swath for application rate of 2.0 gpa and spray atomization of 355 microns mmd (flight 3).

C. Evaluation of Virus Spray Formulation

The satisfactory performance of a water-base virus spray formulation was confirmed. Biological and physiochemical properties of the spray formulation were found to be highly desirable for aerial application. This formulation was not repellent to insect feeding on foliage, and was compatible with the virus and the fluorescent tracer. This formulation is also rain-fast and costs about 7 cents per gallon excluding virus and fluorescent tracer (Maksymiuk, 1968). During these limited tests, no shortcomings of the formulation were detected.

No studies have yet been made on rate of evaporation and on the possible protection of the virus from the effect of ultraviolet radiation (Maksymiuk, 1966). However, this was not the purpose of this study.

D. Evaluation of plastic cards for spray deposit assessment

New plastic cards proved highly effective for both qualitative and quantitative assessment of spray deposit. In the past, paper cards were used to assess spray drops and metal plates to wash out the deposit for colorimetric analysis. It was found that the same plastic cards can be used for simultaneous sampling of spray deposit. This eliminates variation between sampling surfaces (cuts down on the number of test flights) and decreases cost. Spray deposit on cards can be directly projected on a screen for quick comparisons or analyses.

satisfactory performance of a [illegible] spray [illegible]

[illegible] physical properties [illegible]

[illegible] tion were found to be [illegible] suitable for aerial application.

[illegible] formulation was [illegible] in insect feeding on foliage, and was

compatible with the virus and the [illegible] effect. This formulation

also [illegible] cents about [illegible] cents per gallon excluding virus

[illegible] trace. ([illegible], 1963). During these [illegible] 1973,

[illegible] of the formulation [illegible]

No studies have yet been made on rate of evaporation and on the

[illegible] protection of the virus from the effect of ultraviolet radiation

([illegible] 1965). However, this was not the [illegible] of this study.

Evaluation of plastic cards in spray deposit assessment

New plastic cards proved highly effective for both qualitative

quantitative assessment of spray deposits. [illegible], paper

were used to assess spray drops and metal [illegible] to wash [illegible]

it for colorimetric analysis. It was found that [illegible]

can be used for simultaneous sampling of spray deposit [illegible]

correlation between sampling surfaces [illegible]

[illegible] and [illegible] cost. Spray deposit on [illegible]

[illegible] projected on a screen for quick comparisons of [illegible]

VI. CONCLUSIONS AND RECOMMENDATIONS

1. Results show that the application rate can be reduced tenfold (from 2.0 to 0.2 gpa) without reducing larval mortality by using fine spray atomization. This demonstrates, for the first time, the feasibility of ultra low volume (ULV) application of microbial insecticides.

2. A new water-base spray formulation was highly satisfactory for aerial application and quantitative and qualitative assessment of spray deposit.

3. New plastic cards were superior to the previously used paper Kromekote cards. These plastic cards can be used simultaneously for sampling spray drops and for fluorometric qualitative analysis.

4. It is recommended that similar treatments be tested under forest conditions to confirm the feasibility of ULV application of water-base virus spray formulation for control of the Douglas-fir tussock moth and possibly other insects.

5. It is also recommended that similar tests be conducted on the Corvallis spray test site to improve spray application and assessment techniques, spray equipment, and to test new hypotheses.

This limited study resulted in improvements of techniques and reduction of cost for successful aerial application and assessment of water-base sprays.

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A P P E N D I X

APPENDIX A

MEDIUM FOR REARING SEVERAL LEPIDOPTEROUS LARVAE

Ingredients	Amount to make 1,000 ml of medium
1. Dry Ingredients:	
Casein	62.44 g
Sucrose	62.44 g
Wheat germ	53.52 g
Wesson's salts	17.84 g
Alphacel	8.92 g
Ascorbic acid	7.14 g
Aureomycin (surgical)	0.42 g
2. Add liquid ingredients individually to 1:	
Distilled water	446.03 ml
Choline chloride (10% in water)	17.84 ml
Methyl parahydroxybenzoate (15% in 95% EtOH)	11.89 ml
Potassium hydroxide (4 M)	8.92 ml
Sorbic acid (10% in 95% EtOH)	5.35 ml
Vitamin stock solution	2.97 ml
Linolenic acid (55%)	7.43 ml
3. Blend for 2 minutes (Waring blender, low speed)	
4. Add autoclaved agar to 1 and 2	
Agar	22.30 g
Distilled water	499.55 ml
Autoclave and use slow exhaust. Add to above while hot.	
5. Blend whole diet for 2 minutes and then pour into sterile containers.	

Ingredients for vitamin stock solution:

Niacin	600	mg
Calcium pantothenate	600	mg
Riboflavin	300	mg
Thiamin hydrochloride	150	mg
Pyridoxin hydrochloride	150	mg
Folic acid	150	mg
Biotin	12	mg
Vitamin B_{12}	1.2	mg
Distilled Water	100	ml

Ingredients for other stock solutions:

Potassium hydroxide (4 m) - 112.2 gm KOH in 500 ml water

Methyl p-hydroxybenzoate (15% w/v) - 75 gm in 500 ml 95% ethyl alcohol

Sorbic acid (10% w/v) - 50 gm in 500 ml 95% ethyl alcohol

Choline Chloride (10% w/v) - 50 gm in 500 ml water

(Aureomycin and vitamin stock solutions should be stored in the refrigerator)